

Verticillium Wilt of Chrysanthemums and Its Control

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IN BRIEF

Verticillium wilt is a serious and widespread disease of florists' chrysanthemums. It occurs to some extent in most greenhouses where chrysanthemums are grown, and many varieties, excellent except that they are susceptible to wilt, have been discarded by florists because of this disease.

Growers commonly refer to the malady as the "Seidewitz disease" because the varieties Edwin Seidewitz and White Seidewitz were among the first to become severely affected.

Symptoms of wilt are variable in different varieties. Distinct wilting occurs with some, whereas with other varieties no wilting is evident. Diseased plants are stunted, and their leaves die, starting at the base of the stem and progressing upward. Symptoms of the disease become increasingly severe and are most evident when the plants are in flower. Chrysanthemum plants are seldom killed by *Verticillium* wilt.

The disease as it occurs in Ohio is caused by the fungus *Verticillium dahliae* Klebahn. The organism is a common soil fungus and causes disease in many different plants. It enters chrysanthemum plants through the roots and invades the xylem, or water-conducting vessels, of the roots, stems, and leaves.

Verticillium wilt is disseminated by soil infested with the fungus and by cuttings taken from diseased plants.

Control is effected by soil sterilization and the use of cuttings taken from healthy plants. Healthy plants from which to propagate should be selected in the fall before the blooms are cut. These plants should be forced into rapid growth in late winter and spring and tip cuttings taken from the most vigorous shoots. A high percentage of such cuttings are free of wilt and produce healthy plants if grown in sterilized soil. Varieties vary greatly in susceptibility to wilt, and unless the soil can be sterilized, only resistant varieties should be grown.

VERTICILLIUM WILT OF CHRYSANTHEMUMS AND ITS CONTROL

PAUL E. TILFORD AND HARMON A. RUNNELS

INTRODUCTION

The *Verticillium* disease of florists' chrysanthemums was reported first from New Jersey, in 1923 (4). Since that time it has become widespread in the United States and is known to occur in other countries (3, 5, 8, 10, 11, 12).

In Ohio the disease first came to the attention of the authors in 1933, but it had probably occurred in the State previously. At the present time infected plants can be found in most greenhouses where susceptible varieties of chrysanthemums are grown, and *Verticillium* wilt has become the most serious disease affecting this major floral crop. Some varieties, very good except for their extreme susceptibility to *Verticillium* wilt, have been discarded by florists because of this disease.

Growers commonly refer to the malady as "Seidewitz disease" because the varieties Edwin Seidewitz and White Seidewitz were among the first to become severely affected. "*Verticillium* hadromycosis", meaning the disease resulting from an infection of the water-conducting vessels of the plant by a *Verticillium* fungus, is a term used by some writers for the diseases of plants caused by the vascular *Verticillia*. A more common term often used is "*Verticillium* wilt", and in this bulletin the disease in chrysanthemums will be referred to by this name or simply as "wilt."

Many plants other than chrysanthemums are attacked by the vascular *Verticillia*. Rudolph (10) lists 136 host plants belonging to 38 families and 18 orders of the plant kingdom. Since the publication of Rudolph's paper in 1931, additional hosts have been reported. A list of susceptible hosts would include many shade trees, shrubs, vegetables, field crops, flowers, and weeds. The present bulletin deals only with the disease as it occurs in florists' chrysanthemums and gives the results of experiments made to devise practical control measures for the disease. The results of some studies on the causal fungus are reported also.

THE DISEASE

SYMPTOMS

The symptoms of *Verticillium* wilt vary with different varieties of chrysanthemums, and some varieties are affected more severely than others. A distinct wilting occurs in certain varieties during bright days, but the plants regain turgidity at night; with other varieties no wilting is evident. On plants that show wilting, the leaves become a paler green than normal over the whole plant, and finally the leaves die for a part of the way up the stem, starting at the base and progressing upward (fig. 1).

In the varieties that do not wilt, the first symptom usually is the appearance of pale bronze to yellow and later brown dead areas in the lower leaves. These areas may start in the leaf blade between the veins or at the leaf margins. Many times V-shaped areas die at the margins of the leaves with one

of the main veins at the base of the V. Often the margin of the leaf at a particular point will appear wilted and burned without any appreciable change in color at first. Later the whole leaf dies and withers, and the dying of leaves progresses up the stem.

Leaf symptoms may be confined to the leaves on one side of the plant and in some instances to only one-half of a single leaf. In such cases the leaves on the remainder of the plant appear normal and healthy. This unilateral expression of symptoms results when only a part of the vascular tissue is infected by the *Verticillium* fungus.

Severely diseased plants are always stunted and seldom produce marketable flowers (fig. 2). The dying of leaves and stunting become progressively worse as the plants approach flowering.

When diseased plants are producing rapid vegetative growth early in the season, the stunting and leaf symptoms

may not be so evident, but as the blooming period is approached, symptoms become more and more evident. When vegetative growth is temporarily checked by pinching back the tips of diseased plants in a vegetative state, symptoms usually become visible.

A slight brownish discoloration sometimes can be detected in the xylem or woody part of the stem of a diseased plant when it is cut just below the point of attachment of a leaf showing symptoms of *Verticillium* wilt. In many species of plants this symptom is marked. In chrysanthemums, however, this symptom is too indistinct to be of value in diagnosing the disease.

The different symptoms described occur in varying degrees of intensity on plants of different varieties. On some very susceptible varieties, symptoms are severe, and the plants are valueless from the standpoint of flower production. Other varieties may not be so severely affected, and plants, even though diseased, may produce blooms which are marketable although not of first quality. Very seldom does *Verticillium* wilt kill chrysanthemums.

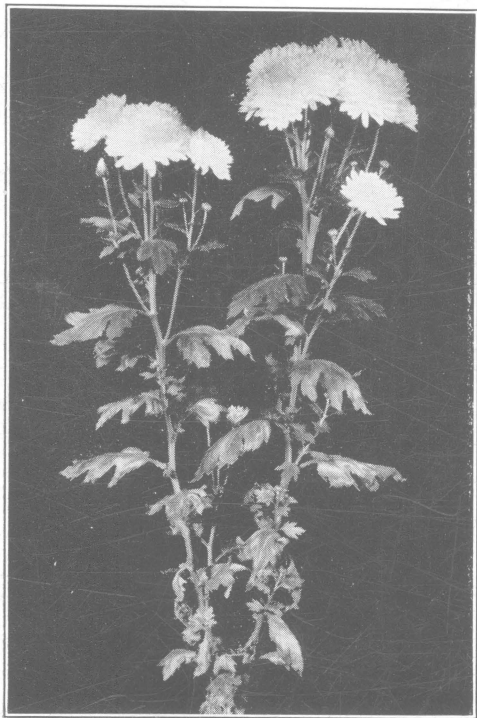


Fig. 1.—Mary Lennon Hall chrysanthemums affected with *Verticillium* wilt, showing wilting, dying, and discoloration of leaves

CAUSE

The disease is caused by the fungus *Verticillium dahliae* Klebahn. The fungus is widely distributed, occurring in the soils of many parts of the world.

Considerable confusion exists in the literature as to the correct taxonomic classification of the *Verticillia* which cause *Verticillium* wilts in different plants. The disagreement is mostly regarding the validity of the species *V. dahliae* Klebahn. This species was described by Klebahn (6) in 1913 and was thought by him to be distinctly different from *V. albo-atrum* R. and B., described earlier by Reinke and Berthold (9). Klebahn believed that the species which he named *dahliae* differed from *albo-atrum* primarily in that it produced distinct sclerotia, whereas *albo-atrum* produced no structures that should be called sclerotia but only dark-colored resting mycelium. Van der Meer (12), Berkeley, Madden, and Willison (1), Ludbrook (7), and Beyma Thoe Kingma (2) support the views of Klebahn. Wollenweber (13) and Rudolph (10) believe that the presence or absence of sclerotia is an unreliable character, not constant enough in the case of this fungus to justify a separate species. They are of the opinion that the two species are synonymous and that the name *albo-atrum* should be used because of priority. Presley (8) has recently reported that he has obtained stable cultures that produce only white mycelium, cultures that produce carbonized, black hyphae, and cultures that produce an abundance of microsclerotia, all from the same monosporic culture of *V. albo-atrum* isolated from chrysanthemums by selective picking of sectors. He expresses the opinion that many of the variations exhibited by the fungus in culture are due to saltation and "..... that most of the 'strains' and 'forms' of the genus are merely variants of the species *albo-atrum* and should be designated as such." No doubt the extreme variability of the vascular *Verticillia*, as well as differences of opinion in the interpretation of the description given by Reinke and Berthold of their fungus, accounts for the disagreement.

Cultures of the fungus isolated from chrysanthemums grow exceptionally well on potato-dextrose-agar media, and in most of our work with the fungus this medium has been used. In newly made transfers the growth is light colored at first and confined mostly to the surface and below the surface of the

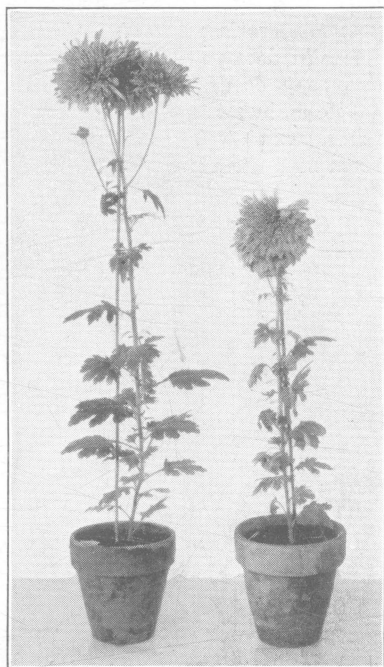


Fig. 2.—Crimson Glow chrysanthemum, affected with wilt on the right; healthy on the left

medium. Within a few days numerous pseudosclerotia¹ form on and in the medium and increase to form a black crust (fig. 3 and 4). In most cultures this crust is dense and continuous over the surface of the colony except for a few millimeters of growth beyond its outer rim. Occasionally sectors will appear in the colonies where the mycelium remains light in color and no pseudosclerotia form (fig. 3). In some instances a definite crust of pseudosclerotia does not form, and in these cultures pseudosclerotia are more sparse and may occur in concentric rings. Some light-colored aerial mycelium develops, but the amount of aerial mycelium varies greatly in different cultures. Conidiophores, typical of the genus *Verticillium*, develop in culture from both the aerial and surface mycelium.

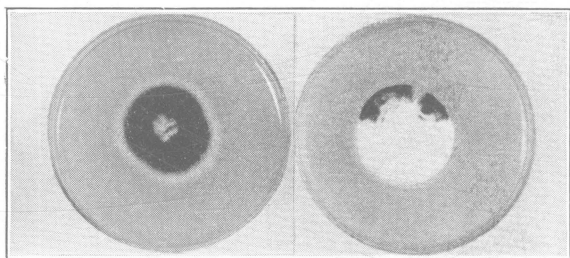


Fig. 3.—Left, typical growth of *V. dahliae* isolated from chrysanthemum. Right, sectoring has occurred and only a part of the growth has produced pseudosclerotia.

Some study has been made of the effect of different factors on the production of pseudosclerotia. Isolates from *Liatris*, silver maple, sugar maple, Norway maple, elm, Japanese barberry, and potato, all of which produce pseudosclerotia, were used in this study along with the chrysanthemum isolates. The length of time that the particular isolate is held in artificial culture, temperature, pH of the medium, and moisture content of the medium produced no visible effect on the ability of the fungus to form pseudosclerotia. The type of inoculum used in making transfers, however, had a marked effect on the time required for pseudosclerotia to form and also on the abundance of these bodies in the subcultures. When the inoculum consisted of a bit of the black pseudosclerotial crust, the subculture usually appeared like the parent culture. However, if only light-colored aerial mycelium or mycelium from a white sector was used for inoculum, the subculture often produced much more white mycelium than the parent culture, and pseudosclerotia, if produced, were slower in forming. White sectors often appeared in cultures started from this type of inoculum, and final pseudosclerotial production was usually more sparse than when some of the crust was used for inoculum. In a few instances cultures were obtained which failed to produce any pseudosclerotia (fig. 5). Upon transfer to fresh media, however, some of these cultures formed pseudosclerotia. Subcultures which remained free of pseudosclerotia for 6 months when grown on potato-dextrose-agar media without being transferred have

¹We have accepted the definition of the term "pseudosclerotia" proposed by Berkeley, Madden, and Willison (1), which is as follows: "Pseudosclerotia: mono-hyphal, thick-walled, dark, coloured, tissue-like formations, the result of a budding process."

been obtained by selecting white mycelium as inoculum from cultures containing pseudosclerotia originally isolated from the following hosts: chrysanthemum, sugar maple, Norway maple, American elm (two isolations), and Japanese barberry. Upon transfer to fresh media, the culture from chrysanthemum produced some pseudosclerotia, but the others have remained free of these structures in subsequent transfers to date. No cultures which remained free of pseudosclerotia for any length of time were secured from the isolations from *Liatris*, silver maple, or potato.

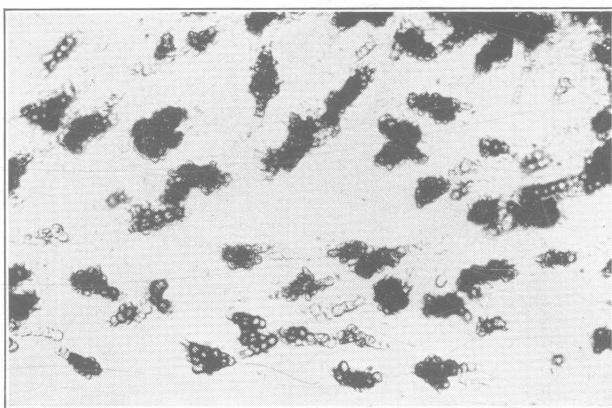


Fig. 4.—Photomicrograph showing pseudosclerotia produced in agar culture media, $\times 200$

In June 1940, rooted, healthy chrysanthemum cuttings of the Mary Lennon Hall variety were planted in sterilized soil and inoculated with each of the five "strains" of *Verticillium* described that had remained white and free of pseudosclerotia. These plants were grown until October, when attempts were made to reisolate the "strains" of the fungus with which the plants had been inoculated. *Verticillium* was reisolated from each set of plants, except the five inoculated with the white selection of the Norway maple "strain." These plants showed no symptoms, and evidently this particular "strain" was not pathogenic. The reisolations were transferred to tubes of potato-dextrose-agar media and kept without transferring from October 1940 to February 1941. After 4 months, three of the reisolations had produced some pseudosclerotia in the culture tubes. One of the "strains" originally isolated from American elm was still free of pseudosclerotia. Evidently passing these white strains through chrysanthemum plants tended to restore their ability to form pseudosclerotia.

Since it had been demonstrated that cultures started from inoculum consisting of light-colored aerial mycelium produced fewer pseudosclerotia than cultures started from inoculum consisting of pseudosclerotial material, it seemed desirable to determine whether their production was governed by genetic factors. If the production of pseudosclerotia is determined by genetic factors, then single spore isolates from some of the cultural types should segregate for production of pseudosclerotia, absence of pseudosclerotia, and

possibly intermediate types. The variability of pseudosclerotial production could then be explained and would not appear to be a suitable basis for species separation.

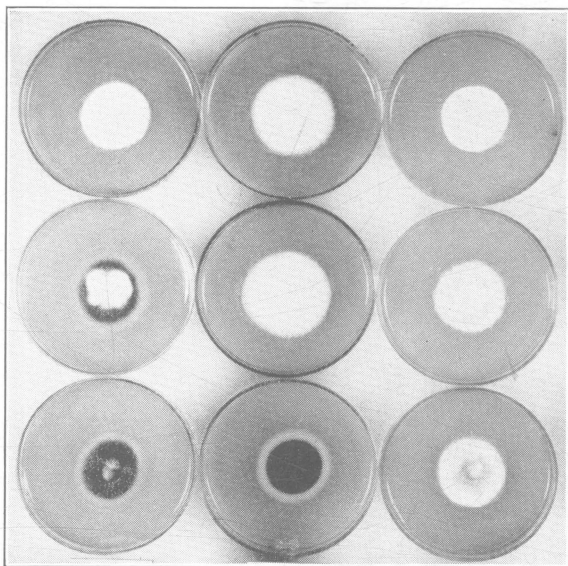


Fig. 5.—Effect of selecting white aerial mycelium for inoculum on subsequent cultures of *V. dahliae*

Left, isolated from sugar maple; center, Japanese barberry; right, American elm. Lower cultures, typical of original isolations. Center and top, cultures obtained after successively selecting only white aerial mycelium as inoculum for several transfers

In June 1941, over 100 single spore isolations were made from a culture of *Verticillium* isolated from the Governor Lake variety of chrysanthemums. Ninety-five of these isolations grew and were carried in culture on potato-dextrose-agar slants for 4 months. Differences in the amount and appearance of aerial mycelium, amount of sectoring, and the abundance of pseudosclerotia were evident, especially in the early stages of growth. Cultures were selected which appeared to be typical of the variations, and single spore subcultures were made of these. Three hundred and thirty-eight such subcultures were obtained. After these had been carried on potato-dextrose-agar for 5 months, by far the majority appeared like the original isolation, in that pseudosclerotial production was abundant, and a black crust was formed. The variations from the normal type exhibited by some of the cultures were differences in the appearance and amount of aerial mycelium and differences in the abundance of, and location of, pseudosclerotia. In some cultures pseudosclerotia tended to form in zones or concentric rings rather than in a continuous layer; in other cultures only a few pseudosclerotia were formed, and these were limited to small patches. Only one culture of the 338 failed to produce any pseudosclerotia.

A culture, designated as *V. albo-atrum* R. & B., was obtained from the American Type Culture Collection for the purpose of comparing it with the *Verticillium* isolated from chrysanthemum. This particular culture bore the number 6481 and according to the data given in the American Type Culture Collection List was isolated from eggplant by J. A. Stevenson and submitted to the collection in 1938. Cultures of *V. albo-atrum* R. & B. and *V. dahliae* Klebahn were secured from the Centraalbureau voor Schimmelcultures at Baarn, Holland.

In our laboratory the *V. albo-atrum* obtained from the American Type Culture Collection occasionally has produced pseudosclerotia when grown in tubes of potato-dextrose-agar media. Usually, however, transfers of this organism have been free of structures which might be interpreted as being pseudosclerotia until the cultures were several weeks old. The *V. albo-atrum* obtained from the Centraalbureau voor Schimmelcultures has not produced any pseudosclerotia while we have had it in culture. Occasionally it has produced small patches of dark-colored mycelium, but no evidence of any group of dark cells resulting from the budding of a single cell has been observed. It appears entirely different from our chrysanthemum isolates. The *V. dahliae* Klebahn obtained from the Centraalbureau voor Schimmelcultures has produced consistently an abundance of pseudosclerotia forming a heavy black crust. This culture appears to be identical with the *Verticillium* we have isolated from chrysanthemums. Inoculation experiments have shown that the *V. dahliae* from Holland and the *V. albo-atrum* from the American Type Culture Collection are both pathogenic to chrysanthemums, but the *V. albo-atrum* from Holland gave negative results in a single inoculation experiment (table 3).

The evidence clearly indicates that the chrysanthemum wilt fungus is similar to the organism *V. dahliae* described by Klebahn (6), in that it produces distinct pseudosclerotia. All the isolations we have made from chrysanthemums, as well as from several other hosts, have definitely produced these structures in culture. Different isolations vary in the amount of pseudosclerotia produced, and both the time required for their formation and the quantity of pseudosclerotia produced can be altered greatly by selecting inoculum from sectors differing in pseudosclerotial production. The ability to produce these structures, however, has remained fairly constant in our cultures and appears to be as stable as many other characteristics used to separate species of fungi.

It is beyond the scope of the present study to determine whether *V. dahliae* and *V. albo-atrum* are or are not synonymous species. The description given by Klebahn for the species *dahliae* fits the chrysanthemum *Verticillium* better than our interpretation of Reinke and Berthold's description of *albo-atrum*. Therefore, it is our conclusion that the fungus causing wilt in florists' chrysanthemums in Ohio should be called *V. dahliae* Klebahn, at least until the confusion which exists regarding the validity of species within the genus *Verticillium* is more satisfactorily cleared up than it is at present.

LIFE HISTORY

If healthy chrysanthemum plants are transplanted to soil that is infested with *Verticillium*, the fungus enters the plants through the roots, primarily through dead or injured roots, and invades the vascular tissue. It progresses upward in the stem and into the leaves by growing in the xylem or water-

conducting vessels. The vascular tissue all around the stem may be infected, or, as is often the case, only the vessels on one side of the stem may be invaded.

The fungus continues to grow upward in the stem at the same time the plant is growing. Often by the time the flowers open, *Verticillium* can be isolated from the top of the stem at the base of the flower.

If diseased plants are saved for stock, the fungus remains alive in these plants over winter. In the spring when cuttings are taken, a high percentage of those obtained from diseased stock plants will carry the fungus and produce diseased plants.

Cuttings from diseased stock plants root readily although the percentage of cuttings that root is usually lower when they are diseased than when they are taken from healthy plants. One hundred cuttings were selected from healthy Mary Lennon Hall plants in May of 1939, and 100 cuttings were selected from diseased plants of the same variety. These were placed in sand in a propagating bench. Three weeks later all 100 of the healthy cuttings had rooted; 83 of those from diseased plants had rooted. The remaining 17 cuttings either had rotted or failed to produce roots. The roots on the cuttings from the diseased plants were noticeably shorter than the roots produced by the healthy cuttings.

DISSEMINATION

The disease is disseminated in two ways, by the use of diseased cuttings and by soil infested with the fungus. Huber and Jones (5) were able to isolate the fungus from cuttings taken from diseased stock plants. Our own work has confirmed this result many times. The results given in table 1 clearly show that diseased stock plants yield a high percentage of diseased cuttings.

TABLE 1.—Disease transmission through stock plants to cuttings

Variety	Number of cuttings taken from diseased stock plants and placed in sterile sand	Number of cuttings rooted	Number of plants diseased*
Andrew Schmidt	9	9	5
Edwin Seidewitz	9	9	6
Mary Lennon Hall.....	285	265	194
Mixed varieties.. ..	135	123	74

*Isolations were made from part of these plants, and the others were grown until symptoms were evident.

Young diseased plants may appear healthy for several weeks following rooting, and all the diseased individuals cannot be discarded when the plants are transplanted to beds or benches. When *Verticillium* wilt is present in the stock plants, the disease is certain to occur in the crop started by propagating from the diseased stock.

The *Verticillium* fungus is a widely distributed soil organism, and when soil is brought into the greenhouse from out-of-doors, it may carry *Verti-*

cillium. Tools that have been used in infested soil may carry contamination to sterilized soil. Also, bits of infested soil clinging to the walls of pots may inoculate susceptible varieties of chrysanthemums grown in pots.

An experiment was set up in the spring of 1939 to determine the extent that healthy plants of a susceptible variety would become diseased in a single season when grown in soil infested with *Verticillium*. Soil in a greenhouse bench was heavily inoculated with *Verticillium*, and healthy rooted cuttings of the very susceptible variety Mary Lennon Hall were set in the bench on May 26. For comparison the soil in another bench was sterilized with steam, and healthy plants were set in this bench. In a third bench the soil was sterilized, but cuttings from diseased stock were used for planting. Yield records and final data were taken on October 30. The results are given in table 2.

TABLE 2.—Effect of *Verticillium* wilt on the Mary Lennon Hall variety of chrysanthemum

Treatment	Number of plants	Number of plants diseased	Average height, inches	Average number of flowering shoots per plant	Total yield, ounces	Average yield per plant, ounces
Sterilized soil Healthy plants.....	36	0	34	6.2	165	4.60
Sterilized soil Diseased plants.....	36	36	18	1.2	17	.47
Inoculated soil Healthy plants.	36	36	20	3.0	33	.92

All the plants that were healthy at the beginning of the experiment became diseased when they were grown in soil infested with *Verticillium*, whereas none of the healthy plants grown in sterilized soil developed wilt. It was noted that the plants which were healthy at the start but became diseased after growing in the inoculated soil yielded almost twice as much as the plants that were diseased at the beginning. It is believed that with some other varieties this difference would be much greater. With the variety Mary Lennon Hall, disease symptoms, including stunting, appear early, whereas with some other varieties symptoms are later in developing. With such varieties the plants would make fair growth before wilt injured them very much, and the yield would be higher. In general, it is believed that healthy plants which become diseased from the soil will outyield considerably plants which were diseased when set in the beds or benches.

SUSCEPTIBILITY OF CHRYSANTHEMUMS TO *VERTICILLIUM* ISOLATED FROM OTHER HOSTS

Since *Verticillium* wilt occurs in so many different kinds of plants, it seemed advisable to determine the susceptibility of chrysanthemums to *Verticillia* isolated from some of the other common hosts. Isolations were made from the following diseased plants found growing in Ohio: *Liatris*, silver maple (*Acer saccharinum*), sugar maple (*Acer saccharum*), Norway maple (*Acer platanoides*), American elm (*Ulmus americana*), Japanese barberry (*Berberis thunbergii*), and potato (*Solanum tuberosum*). Five pots of sterilized soil were inoculated with isolates of *Verticillium* from each of the hosts

named. Two isolations from chrysanthemums were also included. Healthy, rooted cuttings of the variety Mary Lennon Hall were planted in the pots in June 1940 and allowed to grow until September, when notes were taken and the plants were cultured to see whether the fungus could be recovered. In 1941, other experiments were made in a similar manner, and cultures of *V. albo-atrum* from the American Type Culture Collection and *V. dahliae* and *V. albo-atrum*, both from the Centraalbureau voor Schimmelcultures, were included in the 1941 inoculation experiments. The results of these experiments are given in table 3.

TABLE 3.—Results of inoculating Mary Lennon Hall chrysanthemums with different isolations of *Verticillium*

Source of <i>Verticillium</i> inoculum	Number of plants showing symptoms	Severity of symptoms*	Fungus reisolated
Chrysanthemum (Gov. Lake).....	5	+++	+
Chrysanthemum (Ambassador).....	4	+++	+
<i>Liatris</i>	4	+++	+
Silver maple.....	5	+++	+
Sugar maple.....	5	+++	+
Norway maple.....	1	+++	+
American elm.....	1	+++	+
Japanese barberry.....	2	+++	+
Potato.....	5	+++	+
American type culture <i>V. albo-atrum</i>	5	+++	+
Centraalbureau voor Schimmelcultures <i>V. albo-atrum</i>	0	—	—
Centraalbureau voor Schimmelcultures <i>V. dahliae</i>	5	+++	+
Control.....	0	—	—

*Symptoms severe + + +
medium + +
mild +
absent —

It is evident that wilt of chrysanthemums may be caused by the same strains of *Verticillium* that produce disease in several other plants. Undoubtedly *Verticillium* isolated from many other of its host plants would also be pathogenic to chrysanthemums. The occurrence of *Verticillium* wilt is common in many woody and herbaceous plants in Ohio, and apparently there is always the possibility that soil, unless it has been sterilized, is infested with *Verticillium* that is pathogenic to chrysanthemums.

Likewise, *Verticillium* found in diseased chrysanthemums is probably pathogenic to a large number of plants. Soil in beds or benches where chrysanthemums have been grown should not be used for other plants until after it has been thoroughly sterilized.

CONTROL

SOIL STERILIZATION

Four sections of greenhouse bench were filled with soil known to be infested with *Verticillium*. The following treatments were given to the soil in the different bench sections: sterilization with steam, injection of chloropicrin at the rate of 2½ cubic centimeters per square foot, drenching with a 2 per cent formaldehyde solution, and no treatment. The sections treated with chloropicrin and formaldehyde were covered with a 2-inch layer of moist sphagnum for 48 hours following treatment. The soil was then allowed to

aerate for 2 weeks before plants were set in. Eighteen healthy plants of the variety Mary Lennon Hall were set in each section on May 31, 1940. Final data were taken November 4 and are given in table 4.

TABLE 4.—Comparison of methods of sterilizing soil for control of *Verticillium* wilt

Treatment	Number of plants set	Per cent diseased	Average height, inches	Total yield, ounces	Average yield per plant, ounces
Steam	18	0.0	30	75	4.2
Formaldehyde.....	18	5.5	32	77	4.3
Chloropicrin.	18	16.6	30	70	3.9
No treatment	18	100.0	22	25	1.4

All three methods of soil sterilization gave considerable control, but only steam gave complete control. Formaldehyde was satisfactory from the standpoint of control, and the plants in the plot treated with it grew exceptionally well. When steam is unavailable, formaldehyde can be used to free soil of *Verticillium*. When the formaldehyde drench method is used, the soil is completely saturated with the solution. Several days are required for the soil to dry out and for the formaldehyde gas to escape. In addition to this objection, some soils are left in poor physical condition from the puddling effect of so much water. For these reasons the formaldehyde drench method is not nearly as desirable as steam sterilization. Chloropicrin, as used, was slightly inferior to the other methods in control, but it does not have any objectionable effects on the soil.

RESISTANT VARIETIES

Varieties of chrysanthemums differ in susceptibility to wilt. Some are very susceptible; others appear quite resistant. Plants of some varieties are much less likely to become infected with wilt than plants of other varieties even though they are growing in equally infested soil. Certain very susceptible varieties, such as Edwin Seidewitz, have almost gone out of commercial production because of wilt. Some other varieties, grown for years in the same greenhouses with Edwin Seidewitz, have remained relatively free of wilt. The reason for resistance in chrysanthemums to *Verticillium* wilt is not known.

Resistance to wilt is exhibited in two ways: First, plants of some varieties seldom become diseased even though they are grown in *Verticillium*-infested soils and, second, symptoms are slow to develop in plants of some varieties, and the plants are not seriously injured after they become infected. The latter point was demonstrated by an experiment with plants of the resistant variety Good News. Four young healthy plants of this variety were inoculated with *Verticillium* through an incision at the base of the stem in June 1939. After 4 months, when the plants flowered, their average height was 36 inches, and symptoms were confined to the lower leaves on three of the plants. On the fourth plant leaf symptoms were evident on the leaves of one side of the plant on the lower two-thirds of the stem. All the plants produced good marketable flowers. Isolations were made from each of the plants at 3-inch

intervals along the stem to find out how high the fungus had progressed. In the case of the three plants which showed symptoms only on the basal leaves, *Verticillium* was not isolated from points higher on the stems than 9, 12, and 6 inches, respectively, above the point of inoculation. The fungus was recovered from the fourth plant 34 inches above the base. These results are in contrast to those obtained in a similar experiment by using the susceptible variety Andrew Schmidt. The diseased plants of this variety were stunted; symptoms were evident on all the leaves; and the organism was isolated from the very tip of the stems of all five plants cultured.

The four Good News plants used in the experiment described were saved until the spring of 1940 and permitted to produce basal shoots for cuttings. Twenty cuttings from these plants were rooted and transplanted to pots of sterilized soil. All the plants appeared healthy at the end of 8 weeks, and attempts to isolate *Verticillium* from them failed. It is very probable that one of the reasons why *Verticillium* wilt has not become serious in some varieties is that the disease is not readily transmitted by cuttings of these resistant varieties.

There are many greenhouses where it is difficult to sterilize the soil thoroughly. In these houses good crops of resistant varieties can be grown, but when susceptible varieties are used the loss is heavy. There is a distinct need for information on the resistance and susceptibility of varieties so that growers may select the more resistant ones. Chrysanthemum hybridizers are also interested in knowing what varieties are resistant, since in developing new varieties the progeny from resistant parents is more likely to be resistant than are seedlings from susceptible parents. Huber and Jones (5) listed some varieties as being resistant or susceptible, but a more extensive classification is needed.

We have used many varieties² in an experiment to determine resistance and susceptibility. Six rooted cuttings of each variety to be tested were planted in 3-inch pots in soil inoculated with *Verticillium*. The particular strain of *Verticillium* was an isolate from the variety Governor Lake. The fungus was grown on potato-dextrose-agar in Petri plates. When the growth covered the agar it was cut into four equal sections, and one section was placed in each pot around the roots of the plant when it was potted. In a few weeks after inoculation the plants were raised to 4-inch pots and moved out-of-doors to a garden, where the pots were submerged in the soil.

These experiments were started in May 1937 and continued through 1938, 1939, and 1940. About the middle of July, two of the six plants of each variety were cultured for *Verticillium* following the technique described by Huber and Jones (5). If *Verticillium* was isolated from either or both of these two plants, the variety was classed as susceptible. About 1 month later isolations were attempted from two more of the plants in the varieties that did not yield *Verticillium* the first time. Finally, about the middle of September, the last two plants in the varieties which had not yielded *Verticillium* previously were cultured. If the fungus was not isolated from any of the six plants of a variety, the variety was classed as resistant.

This method has been found to give fairly reliable results. However, it is not claimed that any of the varieties classed as resistant are actually immune. In a very few instances some plants of varieties that we have

²The writers wish to thank Yoder Bros., Barberton, Ohio, for furnishing the plants used in this experiment.

classed as resistant have been found affected with wilt in commercial houses. Under some conditions plants of any of the varieties may possibly become infected if the fungus is present in the soil. It is believed, however, that the classification is reasonably accurate. Observations in commercial chrysanthemum plantings and the experiences of growers support it. A previous list of resistant and susceptible varieties was published by the authors (11), but the following lists are much more complete.

Large-flowering commercial varieties.—

Resistant

Betsy Ross	Golden Wave	Okeda
Camilla	Good News	Pink Beauty
Chrysolora	Hilda Bergen	Pink Dawn
Citronella	Honey Dew	Rose Perfection
Columbus Dispatch	Indianola	Silver Sheen
Early Frost	J. W. Prince	Smith's Superlative
Friendly Rival	Major Edward Bowes	Stately White
Golden Glory	Mrs. H. E. Kidder	Windsor Gold
Golden Glow (Improved)	Oak Leaf	

Susceptible

Ambassador	Gold Lode	Nioto
Andrew Schmidt	Golden Arbina	Oconto
Anna Kaskas	Golden Celebration	October Rose
Bonnaffon De Luxe	Golden Mrs. Ross	Old Rose
Cardonia	Golden Oak	Peter John
Chadwick (Bronze, Golden, Pink, White)	Golden Seidewitz	Pink Chief
Chas. Rager	Governor Green	Pink Delight
Chas. W. Johnson	Helen Frick	Pink Treasure
Chattanooga	Immaculate	Quaker Maid
Col. F. M. Alger	Indian Chief	R. B. Mellon
Corona	Indianapolis Pink	Richmond
December Beauty	Justrite	Rose Chochard
Dr. Enguehard	Keystone	Secretary Nehrling
Edwin Seidewitz	Lemon Queen	Silver Dawn
Evening Glow	Lustre	Smith's Early White
Favorite	Major Bonnaffon	Smith's Peerless
Floyd Gibbons	Marie de Petris	Snow White
Friendly Call	Mark Twain	Sunglow
Garnet King	Marketeer	Sun Gold
Glenview	Mefo	Tom Browne
Glitters	Mistletoe (Bronze)	Whittier
	Monument	Yellow Gold
	Mrs. Wm. Thaw, Jr.	

Exhibition varieties.—

Resistant

Alice Benson

Dr. J. M. Inglis

Susceptible

Elberon
Grace Sturgis
J. R. Booth
La France
Louisa Pockett
Majestic
Marian H. Uffinger

Mrs. G. G. Mason
Nagirroc
Rosanda
Turner (William)
Vermont
W. H. Waite
Yellow Pockett

Pompon varieties.—

Resistant

Alice
Angello
Bobbie
Bokhara
Bonnie Maid
Bronze Acto
Chicago Pearl
Clara Jameson
(Improved)
Crystal Jewel
Crystal White
Dainty Maid
Earliwhite
Ecstasy
Elora
Ethel
Fire Bird

Golden Climax
Golden Herald
Golden Spray
Hasegawa Gold
Irene
Jewell
La Vera
Legal Tender
Letitia
Lillian Doty
Long Island Sunshine
Mary Pickford
Masaka
Minong
Mrs. Morgan G. Bulkeley
Nellie Kleris
Nuggets
Olivia

Pink Pearl
Rev. Horace Bushnell
Rising Sun
Robin Hood
Rodell
Romola
Sea Gull
Silver Bells
Silver Tips
Solonore
Source d'Or
Suntan
Thyra
Tokyo
Uvalda
Yellow Doty
Yellow Fellow

Susceptible

Agate
Arcadia
Avalon
Ball of Gold
Betty Watkins
Beuneta
Big Baby
Bonnibell
Bright Spot
Bronze Beauty
Buttercup
Campfire
Capt. Blood
Carlina Lee
Catherine
Cavalcade
Christmas Cheer
Christmas Gold
Comanche
Creole
December Glory
December Gold
Derigold
Dream
Edith Newberry
Elizabeth Peterson
Ermalinda
Fez
Geraldine

Glow
Gold Coin
Gold Drop
Gold Finch
Gold Mine
Gold Tips
Golden Fleece
Golden Nymph
Golden Sceptre
Golden Splendor
Golden Varsity
Governor Lake
Harmony
High Lights
Ida (Improved)
Jemina
Joan
Juva Nicholson
Leileh
Lilac
Lois
Loris
Magatha
Manchukuo
Mary Lennon Hall
Masterpiece
Modena
Mrs. Beu
Mrs. Mary Hooker

Muskoka
Natoma
November Bronze
Nubian
Pagosa
Penguin
Persian Rose
Pink Lassie
Pink Popcorn
Princeton
Rose Charm
Roselea
Rowenna
Royal Crimson
Royal Queen
Silver Ball
Snow Cloud
Stardust
Tom Pearson
Touchdown
Unalga
Usona
Western Beauty
Wildfire
Yellow Bird
Yellow Cordova
Yuletide (White)
Yuvawn

Anemone-flowered varieties.—**Resistant**

Betty Rose
Bronze Volunteer
China Rose
Espy's Dark Pink
Supreme

Estrelita
Millie
Norma
Sunray
White Anemone

Susceptible

Beautiful Lady
Blanche
Captivation
Chrome Emerald
Citrus Queen
Coral Blaze
Crimson Glow
Early Garza Supreme
Eva Le Gallienne
Faith Engel
Fascination

Freida
Garza Supreme
Graceland
Hope Engel
John Shields
Laelia
Little America
Long Island Beauty
Maritza
Noveltie
Orchid Beauty

Red Rolinda
Rolinda
Smith's Innocence
Stoplight
Sunshine
The Belle
The Titan
Topknot
White Izola
Yellow Rolinda
Yolanda

Single, or daisy type, varieties.—**Resistant**

Aloma
Dawn (Hasegawa)
D. Baker
Elizabeth McDowell
Gladys Duckham
Hasegawa Pink

Ida Skiff
Joan Piper
Lighthouse
Mason's Bronze
Sunny Boy

Susceptible

Absolute
Carnelia
Elizabeth Firestone
Golden Gleam
Gretchen Piper
Hasegawa Poinsettia
Helen Hubbard

Joe Beuerlein
Mrs. Darling
Mrs. E. D. Godfrey
Orchid Gem
Pink Pride
Radiant
Red Rover
Ruth Adams

Sunburst
Sunset
Tagoya
Thanksgiving Bronze
Valencia
White Menza
Woburn

Pot plant varieties.—**Resistant**

Greystone

Susceptible

Bright Light
Brutus
Izola
Judith Anderson

Rose Mandel
Titian Beauty
W. H. Lincoln

Hardy varieties.—

Resistant

Aphrodite	Francis Whittlesy	Ruth Cummings
Apollo	Granny Scovill	Ruth Hatton
Barbara Cummings	Gypsy Girl	R. Marion Hatton
Carrie	Indian Summer	Saturn
Ceres	Louis Schling	September Queen
Dazzler	Margot	Wolverine
Ember	Nancy Copeland	Yellow Gem
	Normandie	

Susceptible

A. Barham	Glada	Pink Lustre
Adelaide	Hebe	Provence
Agnes S. Clark	Innocence	Red Flare
Alladin	Jack Bannister	Romany
Amelia	Jean Cummings	Rose Gem
Cavalier	Jean Treadway	Tasiva
Country Girl	Judith Anderson	The Moor
Daphne	King Midas	The Urchin
Diana	Mars	Venus
Eden	Mercury	Vivid
Ganna	Orion	Vulcan

SELECTION OF HEALTHY PLANTS FOR PROPAGATING

Late in the spring of 1937, cuttings of a large number of varieties that were known to contain a high percentage of *Verticillium*-infected plants were selected from plants that appeared healthy. The number of cuttings taken of each variety varied from a few hundred to over a thousand. After rooting, the plants were potted in 4-inch pots of sterilized soil and placed on a greenhouse bench. When the plants were in flower, the following fall, they were inspected, and all plants that showed any evidence of *Verticillium* wilt were discarded. The remaining plants, which appeared healthy, were cut back and transplanted from the pots to a ground bed of sterilized soil. In February 1938, tip cuttings were taken from these plants and rooted. The young plants were set in ground beds adjacent to the original plants of the variety. As soon as new growth on the young plants had developed, tip cuttings were taken and rooted, and the plants were set back in the bed of sterilized soil. This process was repeated several times until a fairly good-sized block of plants was obtained. Some plants developed symptoms of *Verticillium* wilt, and it was evident that all the disease had not been eliminated. When diseased plants did appear they were promptly removed so that cuttings would not be taken from them. Finally, cuttings were taken from the most vigorous plants, rooted, grown in pots for a few days, and then transplanted to ground beds, where they were grown through to flowering.

When the plants were in full flower, October 14, 1938, disease counts were made in some of the varieties. Where it was possible, comparisons were made with plants of the same original stock, handled in the usual way, with no attempt made to eliminate *Verticillium* wilt. The results obtained are given in table 5.

TABLE 5.—Control of *Verticillium* wilt in chrysanthemums by roguing and selection

Varieties	Number of plants in plot	Per cent healthy	Per cent diseased
Bronze Chadwick (rogued and selected)	176	82.4	17.6
Bronze Chadwick (not rogued or selected)	425	9.6	90.4
Pink Chadwick (rogued and selected)	614	94.2	5.8
Pink Chadwick (not rogued or selected)	435	13.8	86.2
Golden Chadwick (rogued and selected)	301	75.4	24.6
Golden Chadwick (not rogued or selected)	443	10.1	89.9
Apricot Queen (rogued and selected)	290	98.3	1.7
Apricot Queen (not rogued or selected)	702	78.8	21.2
October Rose (rogued and selected)	312	71.0	29.0
October Rose (not rogued or selected)	509	67.3	32.7
Crimson Glow (rogued and selected)	48	83.3	16.7
Crimson Glow (not rogued or selected)	129	21.7	78.3
Rose Mandel (rogued and selected)	150	83.4	16.6
Rose Mandel (not rogued or selected)	170	32.4	67.6
White Chief (rogued and selected)	183	79.2	20.8
White Chief (not rogued or selected)	148	20.3	79.7
Indian Chief (rogued and selected)	213	42.3	57.7
Indian Chief (not rogued or selected)	1,350	1.6	98.4

The percentage of diseased plants was greatly reduced by roguing and selection of the stock plants. The practice outlined in this experiment would seldom be advisable for the commercial grower, however. If a grower's stock becomes as highly diseased as this stock was, it should be discarded, and healthy planting stock should be obtained. Ordinarily if only a small percentage of the plants is diseased (10 per cent or less), the plantings should be inspected when the plants are in flower, and healthy individuals should be marked to be saved as propagating stock for the following year. The healthy plants selected should be surrounded by healthy plants and not be adjacent to diseased plants. After the flower crop is removed the marked plants should be lifted and transplanted to the place where they are to be carried through the winter.

Dimock (3) has described a method whereby plants can be obtained that are free of *Verticillium* wilt. Cuttings are selected, and each individual cutting is tagged and given a number. A small piece is removed from the base of each cutting, and this piece is cultured to determine whether *Verticillium* is present. The cuttings are wrapped to prevent drying and stored in a refrigerator. After a week the culture plates are examined, and the numbers of cuttings which yield *Verticillium* are noted. Then the cuttings are removed from the refrigerator; the diseased ones are discarded; and the remainder are placed in sand to root. After healthy, rooted cuttings are obtained they are grown in sterilized soil, and other cuttings can be taken from the tips with assurance that they will produce healthy plants. In this way the stock of healthy plants can be built up fairly rapidly from the original cultured cuttings. The method works very well, but, of course, cannot be used by the ordinary grower, since laboratory facilities are necessary. One large producer of chrysanthemum plants whom the authors know has equipped a plant pathological laboratory and employed a plant pathologist to carry on the work. The results are apparently very satisfactory.

THERAPEUTIC MEASURES

Since *Verticillium* is a vascular parasite, invading the xylem tissue, it would seem that it might be possible to eliminate the fungus from cuttings by therapeutic means. If some material could be found that would be toxic to the fungus and not to the plant tissues, perhaps the cuttings would take up this material when the severed ends were placed in a solution of it, and the fungus would be killed, but the cutting could still be rooted and would produce a healthy plant. With this idea in mind experiments were conducted to test the possibility.

The two materials first tried were the organic mercury preparations, Metaphen and Merthiolate, which are used as disinfectants in the treatment of animal wounds. Cuttings placed in solutions of each of these materials at a dilution of 1-2,500 for 2, 4, 6, and 23 hours were killed. None of the cuttings rooted after the treatment. Since Metaphen is commonly used at a concentration of 1-2,500 and Merthiolate at a concentration of 1-1,000 as antiseptic solutions and these concentrations were lethal to cuttings, no further work was done with these materials.

A number of experiments were made with the chemical 8-hydroxyquinoline sulfate. Tests were first made to determine what concentration of this chemical would be toxic to *V. dahliae* in culture media. Fifty cubic centimeters of potato broth containing 2 per cent dextrose were sterilized in 150-cubic centimeter flasks plugged with cotton. Solutions of 8-hydroxyquinoline sulfate were prepared with sterile water in concentrations of 1 to 100, 1 to 1,000, and 1 to 10,000. Sterile pipettes were used to remove the necessary amount of broth from each flask and then add an equivalent number of cubic centimeters of the proper 8-hydroxyquinoline sulfate solution to give the desired concentration. Triplicate flasks were prepared for each concentration. The concentrations ranged from 0.1 to 500 parts per million. On February 3, 1938, the flasks were inoculated with 2-millimeter plugs of inoculum cut from plate cultures of *V. dahliae* with a cork borer. The cultures were incubated at room temperature for 16 days, and then the relative amount of growth was determined by inspection at the various concentrations. The results are given

TABLE 6.—Growth of *V. dahliae* inoculum in potato-dextrose medium containing 8-hydroxyquinoline sulfate after 16 days and then after being transferred to potato-dextrose-agar

Concentration of 8-hydroxyquinoline sulfate, parts per million	Amount of growth*	Growth after transferring to potato-dextrose-agar	
		Number of cultures growing	Number of cultures not growing
500.....	—	0	3
250.....	—	0	3
175.....	—	0	3
100.....	—	0	3
50.....	—	0	3
25.....	—	0	3
10.....	—	1	2
5.....	—	0	3
2.5.....	—	2	1
1.0.....	—	3	0
.5.....	+	3	0
.25.....	++	3	0
.1.....	++	3	0
0.....	++	3	0

* —, no growth; +, slight growth; ++, good growth.

in table 6. The inoculum was removed from the flasks, where no visible growth occurred, washed in sterile water, and transferred to potato-dextrose-agar media slants. These slants were incubated for 3 weeks to see whether the fungus had been killed. The results are also given in table 6.

The chemical 8-hydroxyquinoline sulfate proved extremely fungistatic to *V. dahliae*. One part per million inhibited growth entirely, but the fungus was not killed until a concentration of 25 parts per million was reached. The inoculum held in this concentration and all higher concentrations for the 16-day period was dead at the end of the experiment. Other similar experiments were made using 8-hydroxyquinoline sulfate, and the results were all essentially the same as those presented in table 6.

In order to obtain information on the time required for different concentrations of 8-hydroxyquinoline sulfate to kill *V. dahliae*, 2-millimeter inoculum plugs were cut from a plate culture and placed in solutions of different concentrations prepared in sterile potato broth containing 2 per cent dextrose. After given periods of time inoculum plugs were removed from the solutions, washed in sterile water, and transferred to test tubes containing potato-dextrose-agar medium. After incubating for 18 days, the cultures were examined for evidence of growth. In the tubes where no growth developed it was considered that the organism was killed by the concentration of 8-hydroxyquinoline sulfate to which that particular inoculum was subjected for the given period of time. The results obtained are given in table 7.

TABLE 7.—The relationship of time and concentration to toxicity of 8-hydroxyquinoline sulfate to *V. dahliae*

Concentration, parts per million	Growth* after exposure to the chemical for the following number of hours:								
	6	23	35	46	57	70	81	93	105
500.....	—	—	—	—	—	—	—	—	—
250.....	—	—	—	—	—	—	—	—	—
175.....	+	+	+	+	+	+	+	—	—
100.....	+	+	+	+	+	+	+	—	+
50.....	+	+	+	+	+	+	—	—	—
25.....	+	+	+	+	+	+	+	—	—

*+, growth; —, no growth.

Since a concentration of 500 parts per million of the chemical killed the fungus (spores, mycelium, and pseudosclerotia were all present in the inoculum used) in 6 hours, cuttings of chrysanthemums were placed in this concentration for various periods of time and then placed in sand to see whether they would root. It was found that cuttings would root after their bases had remained in the 500 parts per million concentration for 24 hours. Injury occurred at the base of the cuttings, but they produced roots one-half to three-fourths of an inch above the base.

Cuttings were taken from Mary Lennon Hall plants affected with *Verticillium* wilt. Fifty of these were placed in water to serve as a control; 70 were placed in a 500 parts per million concentration of 8-hydroxyquinoline sulfate; and 70 were placed in a concentration of 250 parts per million. The treatments were made in beakers, and the lower 1 inch of the stems was covered with the solutions. After 24 hours the cuttings were removed and placed in sand in a propagating bench. Forty-eight of the checks and 67 of

the cuttings in each treatment rooted. Ten of the rooted cuttings in each lot were cultured, and the remaining ones were placed in pots of sterilized soil. Of the plants cultured, *Verticillium* was isolated from six of the check plants, seven of those treated at 250 parts per million, and five of those treated at 500 parts per million. The percentage of the potted plants which developed symptoms of wilt was about the same regardless of whether the cuttings were treated or not. It was evident that the treatment was of no value in eliminating *Verticillium* from the cuttings.

Experiments similar to those described have been made using Orange Helione, Elgetol, and Sinox, but none of these materials has given any better results than the 8-hydroxyquinoline sulfate. An experiment was made to test the toxicity of sodium sulfanilyl sulfanilate to *V. dahliae* in culture. This material showed no toxicity at a concentration of 1,000 parts per million to the fungus in culture, and it was not tested with cuttings.

The results are discouraging as far as using this method to eliminate *Verticillium* from chrysanthemum cuttings is concerned. Perhaps if the right chemicals were found, the method would prove effective. It may be that the 8-hydroxyquinoline sulfate and other materials used react with the plant tissues and become nontoxic before they reach all the fungus. Another possible explanation for the failure of the method is that some of the infected xylem vessels are plugged by the fungus or by gums formed as a result of the infection. If this is the case, it may be impossible for the toxicant to permeate all the vessels and reach all the fungus.

Even though our results were negative, we do feel that it may be possible to find some chemical that will work. The method is a new approach to the problem, and we believe that it should be investigated further.

DISCUSSION AND SUMMARY OF CONTROL MEASURES

There are two distinct phases in the control of *Verticillium* wilt of chrysanthemums. Disease-free plants must be obtained, and the soil, if infested with the fungus, must be sterilized before the healthy plants are transplanted to it.

If over 10 per cent of the plants in a variety are diseased, it will be best for most growers to discard the stock and obtain healthy plants from another source. If the percentage of diseased plants is low, healthy individuals surrounded by healthy plants should be selected in the fall and marked before the flowers are cut. Symptoms are clearer when the plants are in flower than at any other time. After the flowers are cut, the selected healthy plants can be lifted and saved as stock plants for the following year's crop. In the following spring, tip cuttings should be taken from the most vigorous and healthy appearing shoots. These cuttings should be rooted in fresh or sterilized sand, and then the rooted plants should be grown in sterilized soil. Disease-free plants can be produced by culturing each individual cutting for *Verticillium*, but this method cannot be used by most growers.

The sterilization of soil by steam is more satisfactory than sterilizing by formaldehyde or chloropicrin. In some cases, however, where steaming is not possible, these chemical methods can be used.

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